

Please amend claim 1, cancel claims 2-8 and add claims 45-46.

In the claims

1. (currently amended) An isolated O<sup>6</sup>-alkylguanine-DNA alkyltransferase (AGT) mutant, wherein between 6 and 25 amino acids of the wild type human AGT ~~having the amino acid sequence,~~ obtainable by expression of SEQ ID NO: 1, are substituted by other amino acids; and/or 1 to 5 amino acids out of the continuous chain at one, two or three positions are deleted or added; and/or 1 to 4 amino acids at the N-terminus; or 1 to 40 amino acids at the C-terminus are deleted.

2-8. (cancelled)

9. (previously presented) The AGT mutant according to claim 1 wherein two or more modifications are selected from

(A) Cys62 replacement by Ala or Val;

(B) Gln1 15-Gln1 16 replacement by Ala-Asn, Asn-Asn, Ser-His, Ser-Ser, Pro-Pro, Pro-Ser, Pro-Thr, or Thr-Ser;

(D) Gly1 31-Gly132 / Met134-Arg135 replacement by Val-His / Leu-Arg, Lys-Thr / Leu-Ser, Gln-Val / Leu-Ser, or Met-Thr / Met-Val, or Gly131-Gly132 / Met134 replacement by Val-His / Leu; (E) Cys150-Ser1 51-Ser152 replacement by Asn-Ile-Asn, Pro-Leu-Pro, Pro-Arg-Thr, Ser-Phe-Pro-, or Ser-His-Thr-, or Cys150-Ser151 replacement by Phe-Asn or Arg-Asn, or Cys150 / Ser152 replacement by His / Thr, Leu / Asn, Leu / Asn, Leu / Pro or Pro / Leu, or Cys 150 replacement by Ser or Thr;

(F) Pro 140 / Asn157 / Ser159 replacement by Phe / Arg / Glu, or Pro 140 / Asn157 / Gly160 replacement by Met / Trp / Val, or Asn157 /

Ser159-Gly160 replacement by Gly / Glu-Ala, Gly /Asn-Trp, Pro / Gln-Cys or Gly-Gln-Trp, or Asn157 / Ser159 replacement by Gly / Glu, or Asn157 replacement by Gly or Arg; and

(G) truncation after Gly182; and optionally 1 to 10 additional amino acid modifications.

10. (previously presented) The AGT mutant according to claim 1 wherein three or more modifications are selected from

(A) Cys62 replacement by Ala or Val;

(B) Gln1 15-Gln1 16 replacement by Ala-Asn, Asn-Asn, Ser-His, Ser-Ser, Pro-Pro, Pro-Ser, Pro-Thr, or Thr-Ser;

(C) Lys125 replacement by Ala and Ala127-Arg128 replaced by Thr-Ala;

(D) Gly133 1-Gly132 / Met134-Arg135 replacement by Val-His / Leu-Arg, Lys-Thr / Leu-Ser, Gln-Val / Leu-Ser, or Met-Thr / Met-Val, or Gly131-Gly132 / Met134 replacement by Val-His /Leu;

(E) Cys150-Ser1 51-Ser152 replacement by Asn-Ile-Asn, Pro-Leu-Pro, Pro-Arg-Thr, Ser-Phe-Pro-, or Ser-His-Thr-, or Cys150-Ser151 replacement by Phe-Asn or Arg-Asn, or Cys150 /Ser152 replacement by His / Thr, Leu / Asn, Leu / Asn, Leu / Pro or Pro / Leu, or Cys 150 replacement by Ser or Thr;

(F) Pro 140 / Asn157 / Ser159 replacement by Phe / Arg / Glu, or Pro 140 / Asn157 / Gly160 replacement by Met / Trp / Val, or Asn157 / Ser159-Gly160 replacement by Gly / Glu-Ala, Gly /Asn-Trp, Pro / Gln-Cys or Gly-Gln-Trp, or Asn157 / Ser159 replacement by Gly / Glu, or Asn157 replacement by Gly or Arg; and

(G) truncation after Gly182;  
and optionally 1 to 10 additional amino acid modifications.

11. (previously presented) The AGT mutant according to claim 1 wherein two or more modifications are selected from

- (A) Cys62 replacement by Ala;
  - (B) Gln1 15-Gln1 16 replacement by Ser-His;
  - (D) Gly131-Gly132 / Met134-Arg135 replacement by Lys-Thr / Leu-Ser, or Gly131-Gly132 /Met 134 replacement by Val-His / Leu;
  - (E) Cys150-Ser151-Ser152 replacement by Asn-Ile-Asn, or Cys 150 replacement by Ser or Thr;
  - (F) or Asn157 / Ser159 replacement by Gly / Glu; and
  - (G) truncation after Gly182;
- and optionally 1 to 10 additional amino acid modifications.

12. (previously presented) The AGT mutant according to claim 1 wherein three or more modifications are selected from

- (A) Cys62 replacement by Ala;
  - (B) Gln1 15-Gln1 16 replacement by Ser-His;
  - (C) Lys125 replacement by Ala and Ala127-Arg128 replaced by Thr-Ala;
  - (D) Gly131-Gly132 / Met134-Arg135 replacement by Lys-Thr / Leu-Ser, or Gly131-Gly132 /Met 134 replacement by Val-His / Leu;
  - (E) Cys150-Ser151-Ser152 replacement by Asn-Ile-Asn, or Cys 150 replacement by Ser or Thr;
  - (F) or Asn157 / Ser159 replacement by Gly / Glu; and
  - (G) truncation after Gly182;
- and optionally 1 to 10 additional amino acid modifications.

13. (previously presented) The AGT mutant according to claim 1 wherein three or more modifications are selected from

- (A) Cys62 replacement by Ala;
- (B) Gln1 15-Gln1 16 replacement by Ser-His;

(C) Lys125 replacement by Ala and Ala127-Arg128 replaced by Thr-Ala;

(E) Cys150-Ser151-Ser152 replacement by Asn-Ile-Asn, or Cys 150 replacement by Ser or Thr;

(F) or Asn157 / Ser159 replacement by Gly / Glu; and

(G) truncation after Gly182;

and optionally 1 to 10 additional amino acid modifications.

14. (withdrawn) The AGT mutant according to claim 1 selected from mutants with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Asn157Gly, Ser159Glu, truncated after Gly182; Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Asn157Gly, Ser159Glu; Gln115Ser, Gln16His, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu; and Cys62Ala, Gln115Ser, Gln16His, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182.

15. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Gln115Ser, Gln16His, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182.

16. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Gln115Ser, Gln16His, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally 1 to 10 additional amino acid modifications.

17. (withdrawn) The AGT mutant according to claim 16 with modifications Cys62Ala, Gln1 15Ser, Gln1 16His, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally 3 to 7 additional amino acid modifications.

18. (withdrawn and currently amended) The AGT mutant according to claim 16 with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys 150Ser, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His; Ser150Asn, Ser151Ile, Ser152Asn; Lys8Thr, Lys32Ile, Leu33Phe, Thr127Ala, Ser150Asp, Ser151Gly, Ala154Thr; Lys32Ile, Leu33Phe, Ser150Val, Ser152Arg, Gly153Asp, Ala 1154Asp; Lys32Ile, Leu33Phe, Ser150Gly, Ser151Gly, Ser152Asp, Ala154Asp; Ser150Val, Ala154Asp; Ser150Glu, Ser151Gly, Ser152Glu, Ala154Arg; Lys8Thr, Thr127Ala, Ala154Thr; Lys32Ile, Leu33Phe; Ala154Thr; Leu33Phe; Ser151Gly; Ser150 Asp; Thr127Ala; and Lys32Ile, Leu33Phe, and deletion of Leu34.

19. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Gln1 15Ser, Gln1 16His, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Asn, Ser151 Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally 1 to 10 additional amino acid modifications.

20. (withdrawn) The AGT mutant according to claim 19 with modifications Cys62Ala, Gln1 15Ser, Gln1 16His, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Asn, Ser151 Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally 3 to 7 additional amino acid modifications.

21. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Ser, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His; Ser150Asn, Ser151Ile, Ser152Asn; Lys8Thr, Lys32Ile, Leu33Phe, Thr127Ala, Ser150Asp, Ser151Gly, Ala154Thr; Lys32Ile, Leu33Phe, Ser150Val, Ser152Arg, Gly153Asp, Ala1154Asp; Lys32Ile, Leu33Phe, Ser150Gly, Ser151Gly, Ser152Asp, Ala154Asp; Ser150Val, Ala154Asp; Ser150Glu, Ser151Gly, Ser152Glu, Ala154Arg; Lys8Thr, Thr127Ala, Ala154Thr; Lys32Ile, Leu33Phe; Ala154Thr; Leu33Phe; Ser151Gly; Ser150 Asp; Thr127Ala; and Lys32Ile, Leu33Phe, and deletion of Leu34.

22. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Ser, Asn157Gly, Ser159Glu, truncated after Gly182.

23. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Ser, Asn157Gly, Ser159Glu, truncated after Gly182.

24. (withdrawn) The AGT mutant according to claim 1 with modifications Lys32Ile, Leu33Phe, Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Gly, Ser151Gly, Ser152Asp, Ala154Asp, Asn157Gly, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His; Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and

deletion of Leu34.

25. (withdrawn) The AGT mutant according to claim 1 with modifications Lys32Ile, Leu33Phe, Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Val, Ser152Arg, Gly153Asp, Ala154Asp, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His; Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and deletion of Leu34.

26.(withdrawn) The AGT mutant according to claim 1 with modifications Lys32Ile, Leu33Phe, Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Asn, Ser151 Ile, Ser152Asn, Ala154Thr, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His; Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and deletion of Leu34.

27. (withdrawn) The AGT mutant according to claim 1 with modifications Lys32Ile, Leu33Phe, Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Ser, Ala154Thr, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His; Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and deletion of Leu34.

28. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Lys 1125Ala, Ala127Thr, Arg 128Ala, Cys 150Val, Ser1 52Arg, Gly153Asp, Ala154Asp, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His;

Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and deletion of Leu34.

29. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Gly, Ser151Gly, Ser152Asp, Ala154Asp, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His; Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and deletion of Leu34.

30. (withdrawn) The AGT mutant according to claim 1 with modifications Cys62Ala, Lys1125Ala, Ala127Thr, Arg128Ala, Cys150Glu, Ser151Gly, Ser152Glu, Ala154Arg, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His; Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and deletion of Leu34.

31. (withdrawn) A method for detecting and/or manipulating a protein of interest wherein the protein of interest is incorporated into a fusion protein with an AGT mutant according to claim 1 the AGT fusion protein is contacted with particular AGT substrates carrying a label, and the AGT fusion protein is detected and optionally further manipulated using the label in a system designed for recognising and/or handling the label.

32. (withdrawn) The method according to claim 31 wherein an AGT fusion protein mixture containing the AGT fusion protein of the protein of interest and the AGT mutant and a further AGT fusion protein is contacted with a particular substrate, for which either the AGT mutant or the further AGT is selective, the mixture is treated with a further

substrate, and the AGT fusion protein of the protein of interest and the AGT mutant is detected and optionally further manipulated using the label in a system designed for recognising and/or handling the label.

33. (withdrawn) The method according to claim 32 wherein the further substrate is added to the AGT fusion protein mixture after complete reaction of the mixture with the particular substrate.

34. (withdrawn) The method according to claim 32 wherein the further substrate is added to the AGT fusion protein mixture together with the particular substrate.

35. (withdrawn) The method according to claim 34 wherein, in the system designed for recognising and/or handling the label, the label of the particular substrate interacts with the label of the further substrate.

36. (withdrawn) The method according to claim 35 wherein the label of the particular substrate and the label of the further substrate are compounds of a fluorescence resonance energy transfer pair (FRET) or one fluorophore and one quencher for a proximity assay.

37. (previously presented) An AGT fusion protein comprising an AGT mutant according to claim 1 and a protein of interest.

38. (previously presented) The AGT mutant according to claim 1 wherein, when compared to the wild type human AGT, two or more advantageous properties selected from

(a) reduced DNA interaction;

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus;

(c) improved expression yield as soluble protein and improved stability in various hosts;

(d) improved stability under oxidising conditions;

(e) improved stability within cells after reaction with a substrate;

(f) improved stability outside cells before and after reaction with a substrate;

(g) improved *in vitro* solubility;

(h) improved reactivity against 0 6-alkylguanine substrates;

(i) reduced reactivity against DNA-based substrates; and

(j) reduced reactivity against N9-substituted 0 6-alkylguanine substrates, are observed.

39. (previously presented) The AGT mutant according to claim 38 wherein the advantageous properties are

(c) improved expression yield as soluble protein and improved stability in various hosts and

(h) improved reactivity against 0 6-alkylguanine substrates;

or

(c) improved expression yield as soluble protein and improved stability in various hosts,

(d) improved stability under oxidising conditions,

(g) improved *in vitro* solubility, and

(h) improved reactivity against 0 6-alkylguanine substrates;

or

(c) improved expression yield as soluble protein and improved stability in various hosts,

(d) improved stability under oxidising conditions,

(f) improved stability outside cells before and after reaction with a substrate,

(g) improved *in vitro* solubility, and

(h) improved reactivity against O 6-alkylguanine substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) improved expression yield as soluble protein and improved stability in various hosts,

(h) improved reactivity against O 6-alkylguanine substrates, and

(i) reduced reactivity against DNA-based substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) improved expression yield as soluble protein and improved stability in various hosts,

(e) improved stability within cells after reaction with a substrate,

(h) improved reactivity against O 6-alkylguanine substrates, and

(i) reduced reactivity against DNA-based substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) improved expression yield as soluble protein and improved stability in various hosts,

(h) improved reactivity against O 6-alkylguanine substrates,

(i) reduced reactivity against DNA-based substrates, and

(j) reduced reactivity against N9-substituted O 6-alkylguanine substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) improved expression yield as soluble protein and improved stability in various hosts,

(e) improved stability within cells after reaction with a substrate,

(h) improved reactivity against O 6-alkylguanine substrates,

(i) reduced reactivity against DNA-based substrates, and

(j) reduced reactivity against N9-substituted O6-alkylguanine substrates;

40. (previously presented) The AGT mutant according to claim 38 wherein the advantageous properties are

(c') improved expression yield as soluble protein and improved stability in *E. coli* and

(h) improved reactivity against O 6-alkylguanine substrates;

or

(c') improved expression yield as soluble protein and improved stability in *E. coli*,

(d) improved stability under oxidising conditions,

(g) improved *in vitro* solubility, and

(h) improved reactivity against O 6-alkylguanine substrates;

or

(c') improved expression yield as soluble protein and improved stability in *E. coli*,

(d) improved stability under oxidising conditions,

(f') improved stability outside cells after reaction with a substrate,

(g) improved *in vitro* solubility, and

(h) improved reactivity against O 6-alkylguanine substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') improved expression yield as soluble protein and improved stability in *E. coli*,

(h) improved reactivity against O 6-alkylguanine substrates, and

(i) reduced reactivity against DNA-based substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') improved expression yield as soluble protein and improved stability in *E. coli*,

(e) improved stability within cells after reaction with a substrate,

(h) improved reactivity against O 6-alkylguanine substrates, and

(i) reduced reactivity against DNA-based substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') improved expression yield as soluble protein and improved stability in *E. coli*,

(h) improved reactivity against O 6-alkylguanine substrates,

(i) reduced reactivity against DNA-based substrates, and

(j) reduced reactivity against N9-substituted O 6-alkylguanine substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') improved expression yield as soluble protein and improved stability in *E. coli*,

(e) improved stability within cells after reaction with a substrate,

(h) improved reactivity against O 6-alkylguanine substrates,

(i) reduced reactivity against DNA-based substrates, and

(j) reduced reactivity against N9-substituted O 6-alkylguanine substrates;

41. (previously presented) The AGT mutant according to claim 38 wherein the advantageous properties are

(c) more than fivefold expression yield as soluble protein and improved stability in various hosts and

(h) improved reactivity against O 6-alkylguanine substrates;

or

(c) more than fivefold expression yield as soluble protein and improved stability in various hosts,

(d) more than fivefold stability under oxidising conditions,

(g) more than fivefold *in vitro* solubility, and

(h) more than fivefold reactivity against O 6-alkylguanine substrates;

or

(c) more than fivefold expression yield as soluble protein and improved stability in various hosts,

(d) more than fivefold stability under oxidising conditions,

(f) more than fourfold stability outside cells before and after reaction with a substrate,

(g) more than fivefold *in vitro* solubility, and

(h) improved reactivity against 0 6-alkylguanine substrates;

or

(a) less than 2% of DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) more than fivefold expression yield as soluble protein and improved stability in various hosts,

(h) more than fivefold reactivity against 0 6-alkylguanine substrates, and

(i) less than 1% reactivity against DNA-based substrates;

or

(a) less than 2% of DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) more than fivefold expression yield as soluble protein and improved stability in various hosts,

(e) more than threefold stability within cells after reaction with a substrate,

(h) more than fivefold reactivity against 0 6-alkylguanine substrates, and

(i) less than 1% reactivity against DNA-based substrates;

or

(a) less than 2% of DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) more than fivefold expression yield as soluble protein and improved stability in various hosts,

(h) more than fivefold reactivity against O 6-alkylguanine substrates,

(i) less than 1% reactivity against DNA-based substrates, and

(j) less than 2% reactivity against N9-substituted O 6-alkylguanine substrates;

or

(a) less than 2% of DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) more than fivefold expression yield as soluble protein and improved stability in various hosts,

(e) more than threefold stability within cells after reaction with a substrate,

(h) more than fivefold reactivity against O6-alkylguanine substrates,

(i) less than 1% reactivity against DNA-based substrates, and

(j) less than 2% reactivity against N9-substituted O 6-alkylguanine substrates;

42. (previously presented) The AGT mutant according to claim 38 wherein the advantageous properties are

(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli* and

(h) improved reactivity against O 6-alkylguanine substrates;

or

(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,

(d) more than fivefold stability under oxidising conditions,

(g) more than fivefold *in vitro* solubility, and

(h) more than fivefold reactivity against O 6-alkylguanine substrates;

or

(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,

(d) more than fivefold stability under oxidising conditions,

(f') more than fourfold stability outside cells after reaction with a substrate,

(g) more than fivefold *in vitro* solubility, and

(h) improved reactivity against O 6-alkylguanine substrates;

or

(a) less than 2% of DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,

(h) more than fivefold reactivity against O 6-alkylguanine substrates, and

(i) less than 1% reactivity against DNA-based substrates;

or

(a) less than 2% of DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,

(e) more than threefold stability within cells after reaction with a substrate,

(h) more than fivefold reactivity against O6-alkylguanine substrates, and

(i) less than 1% reactivity against DNA-based substrates;

or

- (a) less than 2% of DNA binding,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- (c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,
- (h) more than fivefold reactivity against O 6-alkylguanine substrates,
- (i) less than 1% reactivity against DNA-based substrates, and
- (j) less than 2% reactivity against N9-substituted O 6-alkylguanine substrates;

or

- (a) less than 2% of DNA binding,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- (c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,
- (e) more than threefold stability within cells after reaction with a substrate,
- (h) more than fivefold reactivity against O 6-alkylguanine substrates,
- (i) less than 1% reactivity against DNA-based substrates, and
- (O) less than 2% reactivity against N9-substituted O 6-alkylguanine substrates;

43. (previously presented) The AGT mutant according to claim 38 wherein the advantageous properties are

- (c) more than tenfold expression yield as soluble protein and improved stability in various hosts,
- (d) more than tenfold stability under oxidising conditions,

(f) more than sixfold stability outside cells before and after reaction with a substrate,

(g) more than tenfold *in vitro* solubility, and

(h) more than tenfold reactivity against 0 6 -alkylguanine substrates;

or

(a) no detectable DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) more than tenfold expression yield as soluble protein and improved stability in various hosts,

(e) more than sixfold stability within cells after reaction with a substrate,

(h) more than tenfold reactivity against 0 6 -alkylguanine substrates, and

(i) no detectable reactivity against DNA-based substrates;

or

(a) no detectable DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) more than tenfold expression yield as soluble protein and improved stability in various hosts,

(e) more than sixfold stability within cells after reaction with a substrate,

(h) more than tenfold reactivity against 0 6 -alkylguanine substrates,

(i) no detectable reactivity against DNA-based substrates, and

(j) no detectable reactivity against N9-substituted 0 6 -alkylguanine substrates;

or

- (a) reduced DNA interaction,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- (c) more than tenfold expression yield as soluble protein and improved stability in various hosts,
- (d) more than tenfold stability under oxidising conditions,
- (e) more than sixfold stability within cells after reaction with a substrate,
- (f) more than sixfold stability outside cells before and after reaction with a substrate,
- (g) more than tenfold *in vitro* solubility,
- (h) more than tenfold reactivity against 0 6 -alkylguanine substrates, and

(i) no detectable reactivity against DNA-based substrates;  
or

- (a) reduced DNA interaction,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- (c) more than tenfold expression yield as soluble protein and improved stability in various hosts,
- (d) more than tenfold stability under oxidising conditions,
- (e) more than sixfold stability within cells after reaction with a substrate,
- (f) more than sixfold stability outside cells before and after reaction with a substrate,
- (g) more than tenfold *in vitro* solubility,
- (h) more than tenfold reactivity against 0 6 -alkylguanine substrates,
- (i) no detectable reactivity against DNA-based substrates, and

(j) no detectable reactivity against N9-substituted 0 6-alkylguanine substrates.

44. (previously presented) The AGT mutant according to claim 38 wherein the advantageous properties are

(c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,

(d) more than tenfold stability under oxidising conditions,

(f') more than sixfold stability outside cells after reaction with a substrate,

(g) more than tenfold *in vitro* solubility, and

(h) more than tenfold reactivity against 0 6 -alkylguanine substrates;

or

(a) no detectable DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,

(e) more than sixfold stability within cells after reaction with a substrate,

(h) more than tenfold reactivity against 0 6 -alkylguanine substrates, and

(i) no detectable reactivity against DNA-based substrates;

or

(a) no detectable DNA binding,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,

(e) more than sixfold stability within cells after reaction with a substrate,

(h) more than tenfold reactivity against 0 6 -alkylguanine substrates,

(i) no detectable reactivity against DNA-based substrates, and

(j) no detectable reactivity against N9-substituted 0 6-alkylguanine substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,

(d) more than tenfold stability under oxidising conditions,

(e) more than sixfold stability within cells after reaction with a substrate,

(f) more than sixfold stability outside cells after reaction with a substrate,

(g) more than tenfold *in vitro* solubility,

(h) more than tenfold reactivity against 0 6 -alkylguanine substrates, and

(i) no detectable reactivity against DNA-based substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,

(d) more than tenfold stability under oxidising conditions,

- (e) more than sixfold stability within cells after reaction with a substrate,
- (f) more than sixfold stability outside cells after reaction with a substrate,
- (g) more than tenfold *in vitro* solubility,
- (h) more than tenfold reactivity against O<sup>6</sup>-alkylguanine substrates,
- (i) no detectable reactivity against DNA-based substrates, and
- (j) no detectable reactivity against N<sup>9</sup>-substituted O<sup>6</sup>-alkylguanine substrates.

45. (new) An isolated AGT mutant of a wild-type protein, wherein two or more of the amino acids in the wild-type protein selected from amino acid residues 125-135 of the protein capable of being expressed by SEQ ID NO: 1 are altered, the isolated AGT mutant optionally comprising: (i) one or more altered amino acid residues selected from residues 157 and 159 in a protein capable of being expressed by SEQ ID NO: 1; (ii) deletion of 1 to 4 amino acids at the N-terminus; and/or (iii) deletion of 1 to 40 amino acids at the C-terminus.
46. (new) An isolated AGT mutant of a wild-type protein, wherein amino acid residue 132 in the wild-type protein capable of being expressed by SEQ ID NO: 1 is altered, the isolated AGT mutant optionally comprising: (i) one or more altered amino acid residues selected from residues 157 and 159 in a protein capable of being expressed by SEQ ID NO: 1; (ii) deletion of 1 to 4 amino acids at the N-terminus; and/or (iii) deletion of 1 to 40 amino acids at the C-terminus.